GROWTH HORMONE AND MESOCOTYL GROWTH

by

J. VAN OVERBEEK (Pasadena, Cal.)

1. *Introduction.*

More than perhaps any other problem in plant physiology the one of the mesocotyl [1]) growth has been subject to unsound and phantastic theories. The early students of the growth phenomena of the Avena seedling all had different opinions. Until about 1927 the mesocotyl was considered as an abnormality, due to the cultivation of the seedlings under unfavorable conditions such as laboratory air contaminated with traces of ethylene, excess of CO_2 in the air, etc. The mesocotyl attracted so much attention (though the coleoptile was the main object of their experiments) because of mere technical reasons. The mesocotyl has a tendency to curve when growing out, causing the coleoptile to curve too. The latter, however, has to be straight to be used in the experiments. Moreover a coleoptile of a seedling with a long mesocotyl is always shorter and less sturdy than a coleoptile of a seedling of which the mesocotyl is not developed (see fig. 1 and 2).

The mesocotyl growth was considered a normal process by Pisek (1926), Beyer (1927) and Went (1928). Beyer was the first one who found that by exposing the young plants to light he could grow seedlings without a mesocotyl. Furthermore he discovered the fact that decapitation of the coleoptile tip inhibits the growth of the mesocotyl. An explanation of these facts was given when Went developed the growth substance theory. According to this theory, which is firmly based upon experimental data, the growth of the basal parts of the seedling of Avena (i.e. the mesocotyl and the basal part of the coleoptile) is limited by the amount of growth hormone with which it is supplied. Any decrease in this amount of hormone therefore will result in a decreased growth of the mesocotyl. Now decapitation and exposure to light are both

[1]) The plumula of the seedling of grasses consists of two parts. A basal part or mesocotyl and an apical part or coleoptile. The coleoptile is hollow and envelops the primary leaf. In older publications the expression hypocotyl was used for mesocotyl.

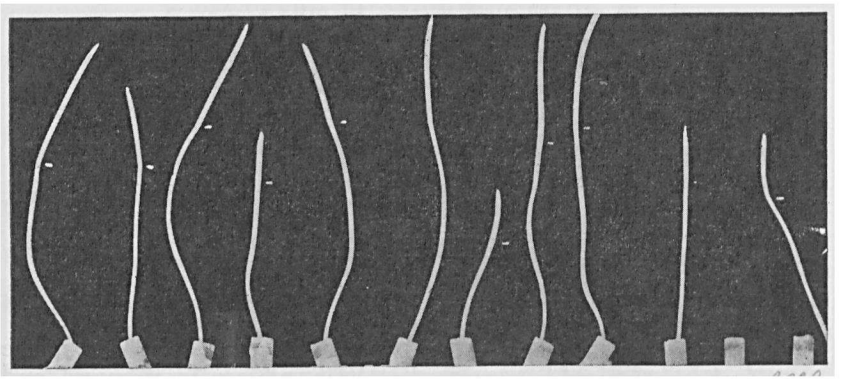

Fig. 1. Avena seedlings grown in water culture in complete darkness. The white lines indicate the place where the mesocotyl changes into the coleoptile.

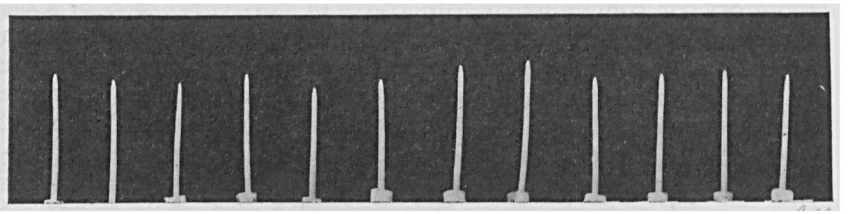

Fig. 2. Avena seedlings grown in water cultures unprotected from the yellow darkroom illumination. The picture only shows the coleoptiles since the mesocotyls are very small and hidden in the glass holders.

procedures that decrease the amount of hormone in the plants, which explains the results of Beyer's experiments (Went 1928, p. 76).

Another way to prevent the mesocotyl from growing out was published by Du Buy and Nuernbergk (1929a). Their method is to expose the plants to heat rays. In two other papers (1929 and 1930) they show that light, from which the heat rays are cut out, is able to inhibit the growth of the mesocotyl. Red light proved to be the most effective and blue the least. While they failed to interpret their results in terms of the above mentioned growth substance theory, they develop another theory which unfortunately is not based upon experimental evidence.

2. *The relation between the amount of growth hormone given off by the coleoptile and the growth of the mesocotyl.*

If young maize seedlings, growing in the dark at about 25°C, were placed for ½ hour in an electric oven in which the temperature of the air was about 48° C the growth of the mesocotyl is strongly inhibited. The final length of these mesocotyls always is shorter than that of the controls (table 2). In order to investigate whether these results can be explained in terms of the theory developed by Went, the following experiments were made. At various times after the plants had been removed from the electric oven, the tips of the coleoptiles were cut off and placed on plain agar blocks for 1½ hour, in order to measure the amount of growth hormone given off by these tips. Table 1 shows that *from 1 to 24 hours after the heat treatment the amount of hormone given off by the coleoptile tips is markedly less than that of the non-treated controls.*

Table 1.

Amount of growth hormone given off by coleoptile tips of corn. Mean values of 24 plants.

No	Time after treatment: 1	2½	6	24 h.	Non-treated
50521	1.5	—	3.2	4.8	7.4
50412	—	2.3	—	—	8.5

If, however, this decreased amount of growth hormone after heat treatment is the cause of the decreased growth of the mesocotyl, an extra amount of hormone applied to the plant immediately after the heat treatment should check this decreased growth. Table 2 shows the results of such an experiment. When the corn seedlings were 3 days old they were placed in the oven for ¾ hour. On the tips of 12 of these plants about 50 mg of a lanolin paste containing 0.02% hetero-auxin was smeared after the heat treatment. When the plants were 5 days old (2 days after the treatment) they were measured.

Table 2.

	Length of		
	coleoptile	primary leaf	mesocotyl
Non-treated, no hormone	30.1	31.0	48.5
Heat treated, no hormone	27.6	34.6	20.7
Heat treated, with hormone	42.7	24.1	48.2

In the last column of this table the figures (mean values of 12 plants)[2]) for the lengths of the mesocotyls are given. *The heat treated mesocotyls are more than 50% shorter than the non-treated controls. If, however, immediately after the heat treatment growth hormone was applied the length of the mesocotyl was the same as in the non-treated controls.* The table also shows the lengths of the coleoptile and the primary leaf of these plants. In the heat treated plants the length of the coleoptile is slightly smaller than in the controls. Application of hormone after the treatment increases the length of the coleoptile considerably. The length of the primary leaf is smallest in the plants that were treated and to which hormone was applied, and largest in the treated plants without an extra hormone supply. In other words the longer the mesocotyl and coleoptile, the shorter the length of the primary leaf. The fact that plants with a very long mesocotyl have a short primary leaf was already noticed by Beyer (1927). Went (1935) in a paper in which he reconsiders his original growth substance theory explains these things fully.

To get an approximate idea to what extent the growth hormone content of the plant changes after the paste has been applied the following experiment was performed. On the tip of the coleoptile of 4 day old corn seedlings about 50 mg of the .02% hetero-auxin paste was smeared. Twelve hours later one set of these plants was cut off 7 mm above the node between coleoptile and mesocotyl; another set of plants was cut off 7 mm below this node. The cut surfaces of these plants were brought into contact with plain agar blocks. After the blocks had been 1 hour in contact with the basal cut surface of the plants they were removed and replaced by new ones, which also were left one hour in contact with the plant. The length of the coleoptiles was 35 mm. If the growth hormone content of these blocks was measured, the plants with the extra supply of hormone gave off an amount of hormone which was about twice that of the normal controls. Table 3 shows the results (experiment number 50528).

The values 14.7 and 13.2 are not maximum angles, as a test, in which the hormone content of the blocks was diluted to half the original concentration, showed.

The growth hormone application in paste form seems a rather inaccurate procedure because the amounts of paste applied are not

[2]) The figures in the tables are all mean values of 12 plants, unless the contrary is stated.

Table 3.

	Amount of g.s. given off during	
	first hour	second hour
Control, cut of above node	7.2	4.7
Control, cut off below node	5.1	3.3
Extra g.s., cut off above node	14.7	13.2
Extra g.s, cut off below node	9.0	7.0

measured out but only estimated. In order to investigate the relation between the amount of paste applied and the amount of hormone given off by the plant the following experiment was made. On top of the coleoptile tips of 4 day old corn seedlings 10, 50 and 300 mg of paste was applied. Twelve hours later these plants were cut off about 7 mm above the node and the amount of g.s. given off by these plants in three consecutive periods of ¾ hour was tested. The results of this experiment (No. 50529) is given in the next table.

Table 4.

	Amount of g.s. given off during		
	first period	second period	third period
10 mg paste applied	12.4 (6.2)	12.2 (6.0)	— (6.3)
50 mg paste applied	— —	14.0 (7.6)	14.3 (6.5)
300 mg paste applied	12.3 (8.8)	14.4 (8.5)	12.5 (7.0)

The figures in parentheses in this table are the values obtained in a test in which the original amount of g.s. in the receiving blocks was diluted once. The table shows clearly that *even large differences in the amount of hormone applied do not affect to any great extent the amount of hormone given off by the plants.*

3. The growth of the mesocotyl of Avena in earth and water cultures.

A phenomenon well known to those who have worked with etiolated seedlings of Avena, is that seedlings grown in earth or sand develop a larger mesocotyl than the ones that develop in the standard glass holders. Two main differences in the way in which the young seedlings are treated can be observed between the methods mentioned above. 1. In earth or sand cultures the roots of the seedlings are in earth or sand, while in the water cul-

tures the roots are submerged in water. 2. The young seedlings in the earth and sand cultures are during the first 1½ day of their development completely buried under the material in which they are grown. During this stage they are therefore shielded from the darkroom illumination. The plants growing up in glass holders are exposed to the darkroom illumination. Du Buy and Nuernbergk (1930) believe that the difference in respiration between the roots in the water cultures and the sand cultures cause the differences in mesocotyl development. That the roots do not influence to any great extent the mesocotyl growth was shown by the following experiment. Seeds were soaked in the usual way for about 1 hour. Some of these seeds were buried in sand, and some other seeds were stuck into glass holders. In the latter case tiny strings of cotton were hung from the seeds into water below the holders in order to moisten the seeds. Then both sets of plants were put into the darkroom in a way that *shielded the plants completely from the darkroom illumination.* Some of the seeds that were buried in the sand were put into the darkroom unshielded from the darkroom illumination also. Four days later the lengths of the coleoptile and mesocotyl were measured. The result is shown in table 5. The figures are the mean values of 20 plants. The number of the experiment is 51110.

Table 5.

	Length of	
	coleoptile	mesocotyl
Water culture, shielded	22.4 mm	56.8 mm
Sand culture, shielded	28.9	60.6
Sand culture, not shielded	39.2	11.2

While the plants growing up in glass holders and not shielded from the darkroom illumination develop a mesocotyl of about 2 mm long, as is known to every one who has made a standard test for growth hormone, *the ones growing up under the same conditions but shielded from the darkroom illumination develop a mesocotyl which is 25 times as long.* Fig. 1 shows the silhouettes of seedlings grown in water culture in complete darkness. Only one plant of the whole set is reasonably straight; all the rest are crooked demonstrating clearly the difficulty that this mesocotyl must have presented to the older investigators. Fig. 2 shows the non-shielded controls.

Another fact in favor of the above point of view is that if

seeds are stuck very closely near the surface of sand and the boxes in which the seeds are planted are not shielded from the darkroom illumination, plants develop with a mesocotyl similar to that of the plants in the water cultures.

That *exposure of the plants to the darkroom illumination causes a decreased growth hormone content* was shown in the following way. Avena seedlings were grown in sand and shielded from the darkroom illumination; another set was grown in the same way but not shielded. When about 4 days old the plants were decapitated and the tips were placed on blocks of plain agar. The preparation of the shielded plants ocurred in very dim (red) light. The unshielded plants were during 3 hours before the tip was cut off exposed to the lamp that illuminates the dark room. This lamp radiates light (and heat) of wave lengths not smaller than 575 m μ. The tips were 2½ hour in contact with the blocks. The analysis of the g.s. content of the blocks showed that the exposed plants had given off 5.4°, while the shielded ones had given off 8.0° (mean values of 40 tips).

In this article the relation of the amount of hormone given off by the tip of the coleoptile is considered in relation with the mesocotyl growth. Other factors which do not act primarily, however, affect the mesocotyl growth also. One of these secondary factors is the „aging" of the plants caused by lack of g.s. (see Went, 1935). If the response to growth hormone is measured about 1 hour after a heat treatment this is found to be the same as in the controls. If it is measured, however, about 6 hours or more after the heat treatment the response to growth hormone is smaller in the treated plants than in the controls. As the experimental data on these factors are not completed as yet no further information can be given in this paper.

4. *Summary.*

If young maize seedlings are exposed to 48°C in air for about ½ hour, the growth of the mesocotyl is markedly inhibited. The amount of growth hormone given off by the tips of the coleoptiles also is less in the heat treated plants than in the controls. By applying extra g.s. to the tips of the heat treated plants it could be proved that the inhibition of the mesocotyl growth is due to the decreased amount of growth hormone given off by the tip.

If Avena seedlings are completely shielded from the darkroom illumination (yellow light) they develop very long mesocotyls in water and earth cultures alike.

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William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, California.

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